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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/563,195	TODD ET AL.	
	Examiner	Art Unit	
	NARAYAN K. BHAT	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-38* is/are pending in the application.

4a) Of the above claim(s) *38* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *03 January 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *4/3/2006 & 7/27/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *Notice to Comply*.

DETAILED ACTION

1. Claims 1-38 are pending in this application.

Election/Restrictions

2. Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claims 1-37 are drawn to a method for detecting the presence or level of alkylated cytosine in a sample.

Group II, claim 38 is drawn to a kit comprising one or more reagents and instructions for detecting the presence or level of alkylated cytosine in a sample.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I, claim 1 is drawn to a method for detecting the presence or level of alkylated cytosine in a sample of genomic or mitochondrial double stranded DNA from an individual, the method comprising: (a) obtaining a sample of the double stranded DNA from the individual; (b) converting at least one region of the double stranded DNA to single stranded DNA; (c) reacting a target region of the single stranded DNA from step (b) with at least one enzyme, the enzyme differentially modifying alkylated cytosine and

cytosine; and (d) determining the level of enzymatic modification of the target region by the enzyme. Group II, claim 38 is drawn to a kit for use in a method of detecting the presence or level of alkylated cytosine in a sample, wherein the kit comprises one or more reagents for performing the method and instructions for use.

The common technical feature as defined by Group I, linking groups I –II is a kit comprising one reagent and instructions for detecting the presence of alkylated cytosine are taught by Opdecamp et al (Nucleic acid research, 1992, 20, 171-178), who teaches reagents (EcoR I, HpaII, Buffers) and method step instructions for detecting the presence of alkylated cytosine in a sample of genomic DNA (Abstract, pg. 172, see Materials and Methods section).

Since Opdecamp et al teaches the kit comprising reagents and instructions for using the kit, the technical feature linking group I and II does not constitute a special technical feature as defined by PCT Rule 13.2, because it does not define a contribution over the prior art. Thus, there is no special technical feature linking the recited groups, as would be necessary to fulfill the requirements for unity of invention.

3. Examiner Cho contacted Applicant's representative Myra McCormack on May 18, 2007 and provisional election was made with traverse to prosecute the invention of group I, claims 1-37. Affirmation of this election must be made by the Applicant in replying to this office action.

4. Claim 38 is withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention of group II there being no allowable generic or

linking claim. Applicant timely traversed the restriction (election) requirement by telephone on May 18, 2007.

5. Claims 1-37 are under prosecution.

6. Examiner has changed for the prosecution of this application. Please send future correspondence to Dr. Narayan K. Bhat, art unit 1634.

Specification

Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosure.

7. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the reason(s) set forth on the attached Notice to Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

Specifically, the application fails to comply with CFR 1.821(c)-(f), which states:

(c) Patent applications which contain disclosures of nucleotide and/or amino acid sequences must contain, as a separate part of the disclosure, a paper or compact disc copy (see § 1.52(e)) disclosing the nucleotide and/or amino acid sequences and associated information using the symbols and format in accordance with the requirements of §§ 1.822 and 1.823. This paper or compact disc copy is referred to elsewhere in this subpart as the "Sequence Listing." Each sequence disclosed must appear separately in the "Sequence Listing." Each sequence set forth in the "Sequence Listing" must be assigned a separate sequence identifier. The sequence identifiers must begin with 1 and increase sequentially by integers. If no sequence is present for a sequence identifier, the code "000" must be used in place of the sequence. The response for the numeric identifier <160> must include the total number of SEQ ID NOs, whether followed by a sequence or by the code "000."

(d) Where the description or claims of a patent application discuss a sequence that is set forth in the "Sequence Listing" in accordance with paragraph (c) of this section, reference must be made to the sequence by use of the sequence identifier, preceded by "SEQ ID NO:" in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application.

(e) A copy of the "Sequence Listing" referred to in paragraph (c) of this section must also be submitted in computer readable form (CRF) in accordance with the requirements of § 1.824. The computer readable form must be a copy of the "Sequence Listing" and may not be retained as a part of the patent application file. If the computer readable form of a new application is to be identical with the computer readable form of another application of the applicant on file in the Office, reference may be made to the other application and computer readable form in lieu of filing a duplicate computer readable form in the new application if the computer readable form in the other application was compliant with all of the requirements of this subpart. The new application must be accompanied by a letter making such reference to the other application and computer readable form, both of which shall be completely identified. In the new application, applicant must also request the use of the compliant computer readable "Sequence Listing" that is already on file for the other application and must state that the paper or compact disc copy of the "Sequence Listing" in the new application is identical to the computer readable copy filed for the other application.

(f) In addition to the paper or compact disc copy required by paragraph (c) of this section and the computer readable form required by paragraph (e) of this section, a statement that the "Sequence Listing" content of the paper or compact disc copy and the computer readable copy are the same must be submitted with the computer readable form, e.g., a statement that "the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing."

The Specification discloses nucleotide sequences in Fig. 2A-C and on pages 31, 32 and 33. However, sequences are not identified by "SEQ ID NO."

For compliance with sequence rules, it is necessary to include the sequence in the "Sequence Listing" and identify them with SEQ ID NO. In general, any sequence that is disclosed and/or claimed as a string of particular bases or amino acids, and that otherwise meets the criteria of CFR 1.821(a), must be set forth in the "Sequence Listing." See MPEP 2422.03.

While the Examiner has made every attempt to check the Specification for sequence compliance, Applicant is required to carefully check the entire Specification for any and all issues regarding sequence compliance.

For the response to this Office Action to be complete, Applicant is REQUIRED to comply with the Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures. Failure to comply with the Requirements will be considered nonresponsive.

Claim Rejections - 35 USC § 112

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. Claims 19, 21 and 26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

10. Claim 19 recites the limitation "said enzymes" in line 2. There is insufficient antecedent basis for the "one or more enzymes" of claim 1.

11. Claim 21 is indefinite over the recitation of the enzyme acronyms in line 2 and is confusing because the acronyms used for the enzymes can change with time. It is suggested that full name of enzyme be cited at the first use.

12. Claim 26 is indefinite over the recitation of the gene acronyms in lines 2 and 3 and is confusing because the acronyms used for naming the gene can change with time. It is suggested that full name of the gene be cited at the first use.

Claim Rejections - 35 USC § 102

13. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

14. Claims 1-3, 14, 19, 22-25, 27-33, 35 and 37are rejected under 35 U.S.C. 102(b) as being anticipated by Opdecamp et al (Nucleic Acids Research, 1992, 20, 171-178) as evidenced by Griffith et al (An Introduction to Genetic Analysis, Section 12, pgs. 1-4).

Regarding claim 1, Opdecamp et al teaches a method for detecting the presence or level of alkylated cytosine in a sample of genomic double stranded DNA from an individual, the method comprising: (a) obtaining a sample of the genomic double stranded DNA from the individual (pg. 172, Materials and methods section, paragraphs 1 and 2, step 'a' of the claim) and further teaches converting at least one region of the double stranded DNA to single stranded DNA by digesting with EcoR I restriction enzyme, which generates single stranded sticky ends (Fig. 2, lane marked with E, pg. 172, column 2, Results section, paragraph 1, step 'b' of the claim). Generation of single stranded sticky ends with EcoR I digestion of the genomic DNA is further supported by the teachings of Griffith et al, who teaches the digestion of the genomic DNA with EcoR I leaves the single stranded sticky ends (Griffith et al, section 12, pg. 3). It is noted that, teachings of Griffiths et al of digestion of the genomic DNA with EcoR I to generate single stranded sticky ends, is only used for further supporting a known fact in the art.

Opdecamp et al further teaches reacting a target region of the single stranded DNA from step (b) generated by digesting the genomic DNA with EcoR I further with Hpa II (Fig. 2, lane marked with EH, pg. 172, column 2, Results section, paragraph 1). Opdecamp et al also teaches that Hpa II cuts the DNA containing 'CCGG' DNA

sequence but not the DNA containing inner cytosine methylation "CmCGG" DNA sequence (pg. 172, column 2, Results section, paragraph 1), thus teaching at least one enzyme, the Hpa II enzyme differentially modifying methylated cytosine and cytosine. The methylated cytosine is the alkylated cytosine as defined in the instant claim 35. The cleavage of the DNA by Hpa II enzyme containing recognition sequence 'CCGG' and not cleaving the DNA sequence 'CmCGG' as taught by Opdecamp et al is broadly interpreted as a modification step by the enzyme.

The DNA segment comprising the Hpa II recognition sequence is the target binding region of the claim. Since EcoR I digested DNA comprise single strand sticky ends and has the target region for Hpa II enzyme and therefore meet the limitation of "reacting a target region of the single stranded DNA with at least one enzyme" (limitation of step 'c'). It is noted that the step 'c' as recited in the claim 1, does not require that the enzyme bind directly to the single stranded DNA but rather react with a single strand DNA comprising a target region.

Opdecamp et al also teaches determining the level of Hpa II enzymatic modification of the target region by determining the methylation levels in the target region (Fig. 5, See legend for the methylation levels, Results section, pg. 174 columns 1 and 2 last paragraph).

Regarding claim 2, Opdecamp et al teaches that the DNA comprising single stranded sticky end DNA is reacted with the Hpa II enzyme under conditions such that the enzyme reacts substantially only with cytosine in the single stranded DNA but not

with methylated, i.e., alkylated cytosine (pg. 172, column 2, Results section, paragraph 1).

Regarding claim 3, Opdecamp et al teaches that the DNA comprising single stranded sticky end DNA is reacted with the Hpa II enzyme under conditions such that the enzyme reacts substantially only with cytosine in the single stranded DNA (pg. 172, column 2, Results section, paragraph 1).

Regarding claim 14, Opdecamp et al teaches that the determination of the level of enzymatic modification of the DNA with single stranded sticky ends comprises analyzing for methylated and unmethylated cytosine sequence variations arising from the enzymatic modification of the target region of the single stranded DNA by the enzyme (Fig. 5, see the legend).

Regarding claim 19, Opdecamp et al teaches a combination of EcoR I and Hpa II enzymes are employed to differentially modify alkylated cytosine and cytosine in the target region (Fig. 2, pg. 172, column 2, last paragraph).

Regarding claims 22 and 23, Opdecamp et al teaches detecting the presence of alkylated cytosine in a 5' non-coding region of the gene, i.e., untranslated region of the gene (Fig. 1A, Fig. 2, pg. 173, paragraph 1).

Regarding claim 24, Opdecamp et al teaches that the level of methylated, i.e., alkylated cytosine comprises hypermethylation (pg. 176, column 2, paragraph 3).

Regarding claim 25, Opdecamp et al teaches that the level of methylated, i.e., alkylated cytosine comprises hypomethylation (pg. 176, column 2, paragraph 3).

Regarding claim 27, Opdecamp et al teaches that the detection of an altered level of alkylated cytosine in the target region of the single stranded DNA is different in hepatoma than in adult liver (Fig. 5, Compare the pattern of hepatoma with fetal and adult liver), thus teaching a marker for hepatoma, i.e., disease of the liver.

Regarding claim 28, Opdecamp et al teaches hepatoma, i.e., a tumor of the liver, thus teaching that the disease is cancer (Fig. 5).

Regarding claim 29, Opdecamp et al teaches a hepatoma, i.e., a tumor of the liver, thus teaching that the disease is cancer (Fig. 5), thus teaching the cancer is selected from liver cancer.

Regarding claim 30, Opdecamp et al teaches that the detection of an altered level of alkylated cytosine in the target region of the single stranded DNA is different in hepatoma than in adult liver (Fig. 5, Compare the pattern of hepatoma with fetal and adult liver), thus teaching diagnosing a disease hepatoma on the basis of the presence of alkylated cytosine in the target region of the single stranded DNA.

Regarding claim 31, Opdecamp et al teaches a hepatoma, i.e., a tumor of the liver, thus teaching that the disease is cancer (Fig. 5), thus teaching the cancer is selected from liver cancer.

Regarding claim 32, Opdecamp et al teaches that the detection of an altered level of alkylated cytosine in fetal liver than in adult liver (Fig. 5, Compare the pattern of fetal and adult liver), thus teaching the level of the alkylated cytosine is detected to indicate the presence or absence of fetal DNA.

Regarding claim 33, Opdecamp et al teaches an embodiment wherein methylation status in the AFP gene at site M0 in the adult liver indicates the presence of an altered gene imprinting state (pg. 176, column 2, last paragraph).

Regarding claim 35, Opdecamp et al teaches that the alkylated cytosine is methylated cytosine (pg. 172, column 2, last paragraph).

Regarding claim 37, Opdecamp et al teaches that the double stranded DNA is genomic DNA (pg. 172, paragraph 3).

Claim Rejections - 35 USC § 103

15. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

16. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

17. Claims 1, 4-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Opdecamp et al (Nucleic Acids Research, 1992, 20, 171-178) as evidenced by Griffith et al (An introduction to Genetic Analysis, Section 12, pgs. 1-4) in view of Kuhn et al (J. Am. Chem. Soc., 2002, 124, 1097-1103).

Claim 13 is dependent from claim 12. Claims 9-12 are dependent from claim 8, which is dependent from claim 4, which is dependent from claim 1. The teachings of Opdecamp et al regarding claim 1 are described in section 14 of this office action.

Regarding claim 4, Opdecamp et al teaches the conversion of the region of the double stranded DNA to the single stranded DNA by digesting with EcoR I restriction enzyme, which generates single stranded sticky ends (Fig. 2, lane marked with E, pg. 172, column 2, Results section, paragraph 1). Generation of single stranded sticky ends with EcoR I digestion of the genomic DNA is further supported by the teachings of Griffith et al, who teaches the digestion of the genomic DNA with EcoR I leaves the single stranded sticky ends (Griffith et al, section 12, pg. 3). It is noted that, teachings of Griffiths et al of digestion of the genomic DNA with EcoR I to generate single stranded sticky ends, is only used for further supporting known fact in the art. Opdecamp et al are silent about partially separating the two strands of the double stranded DNA. However, partially separating the two strands of the double stranded DNA with different means including strand displacing probes were known in the art at the time of the claimed invention was made as taught by Kuhn et al.

Regarding claim 4, Kuhn et al teaches a method for opening of the double stranded DNA with PNA openers to partially separate the two strands of the double stranded DNA (Fig. 5A, right panel, pg. 1101, column 1, paragraph 2).

Regarding claim 5, Kuhn et al teaches a method wherein PNA openers (i.e., strand displacing probes) are used to partially separate the two strands of the double stranded DNA (Fig. 5A, right panel, pg. 1101, column 1, paragraph 2).

Regarding claim 6, Kuhn et al teaches that the strand displacing probes are PNA containing probes (Fig. 5A, right panel, pg. 1101, column 1, paragraph 2).

Regarding claim 7, Kuhn et al teaches a method wherein the double stranded DNA is opened with PNA openers and further teaches that PNA openers forms triplexes and exposes the displaced DNA strand for binding with other DNA and PNA beacons (Fig. 5A and B, right panel, pg. 1101, column 1, paragraph 2), thus teaching inhibiting annealing of the two strands of the double stranded DNA together once they have been separated to facilitate access to the target region by the enzyme. With regard to the recitation of "to facilitate access to the target region by the enzyme", the phrase is the property of the DNA being at least partially separated, which Kuhn et al teaches.

Regarding claim 8, Kuhn et al teaches hybridizing at least one PNA beacon probe with a strand of the double stranded DNA following separation of the two strands to form a triplex structure, thereby inhibit the annealing of the two strands together (Fig. 5A and B, right panel, pg. 1101, column 1, paragraph 2).

Regarding claim 9, Kuhn et al teaches that the PNA probe is antisense probe (Fig. 5B, right panel, Fig. 7A, See the legend, pg. 1102, column 2, paragraph 1).

Regarding claim 10, Kuhn et al teaches at least two PNA opener probes are hybridized with the strand of the double stranded DNA, one of the probes hybridizing with a region of the strand downstream of the target region (Fig. 5A, right panel, see the PNA opener at the right side of the displaced strand) and other probe hybridizing with a region of the strand upstream of the target region (Fig. 5A, right panel, see the PNA opener at the left side of the displaced strand).

Regarding claim 11, Kuhn et al teaches wherein the probe hybridizes with upstream and downstream regions of the strand which flank the target region such that a loop which incorporates the target region is formed in the strand (Fig. 5A, right panel, see the loop formed by two PNA openers).

Regarding claim 12, Kuhn et al teaches that the probe hybridizes with the strand of the double stranded DNA either side of the target region (Fig. 5A, right panel) and further teaches that the probe has a middle region of non-complementary sequence that does not hybridize with the target region such that a loop incorporating the target region is formed in the strand (Fig. 5A, right panel).

Regarding claim 13, Kuhn et al teaches that the middle region of the probe incorporates inverted repeats that hybridize together following hybridization of the probe with the strand of the double stranded DNA (Fig. 5A, right panel).

Kuhn et al also teaches that PNA beacons are insensitive to the presence of salt and DNA-binding/processing proteins and have a great potential as robust tools for recognition of specific sequence within double strand DNA without denaturation and deproteinization of duplex DNA (Abstract).

It would have been prima facie obvious to one having the ordinary skill in the art at the time the invention was made to modify the methylation detection method of Opdecamp et al and include the method of using PNA openers for partially separating the two strands of the double stranded DNA of Kuhn et al with a reasonable expectation of success.

An artisan would have been motivated to modify the methylation detection method of Opdecamp et al and include the method of using PNA openers for partially separating the two strands of the double stranded DNA of Kuhn et al with the expected benefit of using PNA beacons, which are insensitive to the presence of salt and DNA-binding/processing proteins and have a great potential as robust tools for recognition of specific sequence within double strand DNA without denaturation and deproteinization of duplex DNA as taught by Kuhn et al (Abstract).

18. Claims 1, 14-17, 22-23 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Opdecamp et al (Nucleic Acids Research, 1992, 20, 171-178) as evidenced by Griffith et al (An introduction to Genetic Analysis, Section 12, pgs. 1-4) in view of Gitan et al (Genome Research, 2001, 12, 158-164).

Claim 17 is dependent from claim 16, which is dependent from claim 15, which is dependent from claim 14. Claim 26 is dependent from claim 23, which is dependent from claim 22. Claims 14 and 22 are dependent from claim 1. The teachings of Opdecamp et al regarding claims 1, 14, 22 and 23 are described in section 14 of this office action.

Regarding claim 15, Opdecamp et al are silent about subjecting the target region of the single stranded DNA to an amplification process involving thermocycling and primers to obtain an amplified product, and analyzing the amplified product for sequence variations. However, amplification of target DNA by PCR was known in the art at the time of the claimed invention was made as taught by Gitan et al, who teach PCR amplification of the selected target region using PCR to obtain an amplified product and analyzing the amplified product by sequence variation (Fig. 1, See the legend for details).

Regarding claim 16, Gitan et al teaches that the analysis of the amplified product comprises subjecting the amplified product to a technique involving the use of probes that bind to specific nucleic acid sequences (Fig. 1, bottom panel).

Regarding claim 17, Gitan et al teaches that the analysis of the amplified product comprises subjecting the amplified product to a polymerase chain reaction technique with nested primers (pg. 162, column 1, paragraph 1).

Regarding claim 26, Gitan et al teaches that the gene is ER alpha (Abstract).

Gitan also teaches that the target amplification provides a method to label the specific target gene with fluorophore and provides method to determine methylation changes in multiple CpG island loci and for generating epigenetic profile in cancer (Fig. 1, Abstract).

It would have been prima facie obvious to one having the ordinary skill in the art at the time the invention was made to modify the methylation detection method of

Opdecamp et al and include the target amplification method of Gitan et al with a reasonable expectation of success.

An artisan would have been motivated to modify the methylation detection method of Opdecamp et al and include the target amplification method of Gitan et al with the expected benefit of labeling the specific target gene with fluorophore and providing method to determine methylation changes in multiple CpG island loci and for generating epigenetic profile in cancer as taught by Gitan et al (Fig. 1, Abstract).

19. Claims 1 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Opdecamp et al (Nucleic Acids Research, 1992, 20, 171-178) as evidenced by Griffith et al (An introduction to Genetic Analysis, Section 12, pgs. 1-4) in view of Paulson et al (J. Virol., 1999, 73, 9959-9968).

Claim 34 is dependent from claim 1. Teachings of Opdecamp et al regarding claim 1 are described in section 14 of this office action.

Regarding claim 34, Opdecamp et al are silent about the presence of alkylated cytosine to indicate the presence or absence of a pathogen or microorganism. However, the presence of alkylated cytosine to indicate the presence or absence of a pathogen or microorganism was known in the art at the time of the claimed invention was made as taught by Paulson et al, who teaches that the EBV is the etiologic agent of infectious mononucleosis, i.e., a pathogen (pg. 9959, column 1, paragraph 1) and further teaches that the presence of EBV is characterized by the presence of its methylated promoter sites (Fig. 3, pg. 9964, column 2 paragraph 2). Paulson et al also teaches that EBV

usurps the host cell directed methylation system to regulate pathogen gene expression and thereby establish a chronic infection (Abstract).

It would have been prima facie obvious to one having the ordinary skill in the art at the time the invention was made to modify the methylation detection method of Opdecamp et al and include the method of detecting methylated promoter of EBV of Paulson et al with a reasonable expectation of success.

An artisan would have been motivated to modify the methylation detection method of Opdecamp et al and include the method of detecting methylated promoter of EBV of Paulson et al with the expected benefit of detecting the pathogen EBV as taught by Paulson et al (Abstract, pg. 9959, column 1, paragraph 1).

20. Claims 1, 18, 20- 21 and 35-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chaudhuri et al (Nature, 2003, 422, 726-730) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107, This reference was cited in the IDS filed 4/3/2006).

Regarding claim 1, Chadhuri et al teaches a transcription –targeted DNA deamination method comprising (a) obtaining a sample of the genomic double stranded DNA from the individual (pg. 729, Methods section, paragraphs 1, step 'a' of the claim). It is noted that the DNA sample comprises the amplified DNA sample from the synthetic insert cloned in E.Coli, which is broadly interpreted as DNA from the individual E. coli. Chaudhuri et al further teaches converting at least one region of the double stranded DNA to single stranded DNA by heating the sample at 100C and chilling on ice (pg. 729, Methods section, paragraphs 1, step 'b' of the claim).

Chaudhuri et al further teaches reacting a target region of the single stranded DNA from step (b) with cytidine deaminase, which converts cytidine in the single stranded substrate to uridine (Fig. 1b, pg. 727, column 2, paragraph 1, pg. 729, Methods section, DNA cytidine deamination assay). Chaudhuri et al teaches that cytidine deaminase is AID enzyme and is single strand specific (Fig. 1b, pg. 727, column 2, paragraph 1, pg. 728, column 1, paragraphs 1-2, pg. 728, column 2, paragraph 1). Chaudhuri et al are silent about differentially modifying alkylated cytosine and cytosine.

Chaudhuri et al also teaches determining the level of cytidine deaminase enzymatic modification of the target region by determining the conversion of cytidine to uridine levels in the target region (Fig. 1b, pg. 727, column 2, paragraph 1, pg. 728, column 1, paragraph 1,pg. 729, Methods section, DNA cytidine deamination assay, step 'd' of the claim).

Regarding claim 18, Chaudhuri et al teaches that AID is a single strand specific enzyme with no activity on double stranded DNA (pg. 728, column 2, paragraph 2) and further teaches that AID deaminates cytosine in the target region of the single stranded DNA (Fig. 1b, See the figure legend for details, pg. 727, column 2, paragraph 1).

Regarding claim 20, Chaudhuri et al teaches that the AID enzyme is independently a deaminase enzyme having deaminase activity (Fig. 1b, see the right most panel, pg. 727, column 2, paragraph 1).

Regarding claim 21, Chaudhuri et al teaches that the enzyme is AID enzyme (Fig. 1b, see the right panel, pg. 727, column 2, paragraph 1).

Regarding claims 35-36, Chaudhuri et al are silent about alkylated cytosine.

Chaudhuri et al are also silent about differentially modifying alkylated cytosine and cytosine. However, an enzyme differentially modifying alkylated cytosine and cytosine was known in the art at the time of the claimed invention was made as taught by Bransteitter et al, who teaches AID enzyme and further teaches AID enzyme modulates the activity of single strand DNA containing cytosine and methylated cytosine, i.e., alkylated cytosine differentially by 10-fold (Fig. 4b, pg. 4106, column 1, paragraph 4), thus teaching AID differentially modifying alkylated cytosine and cytosine.

Regarding claim 35, Bransteitter et al teaches methylated cytosine (pg. 4102, column 2, paragraph1), which is an alkylated cytosine as defined in the instant claim.

Regarding claim 36, Bransteitter et al teaches methylated cytosine is 5-methyl cytosine (pg. 4102, column 2, paragraph1).

Bransteitter et al also teaches that AID enzyme differentially modifies single stranded DNA containing 5-methyl cytosine than cytosine (Fig. 4b, pg. 4106, column 1, paragraph 4) and further teaches that the AID enzyme target the single stranded DNA region at the transcription site to initiate somatic hypermutation (pg. 4106, column 2, paragraph 3).

It would have been prima facie obvious to one having the ordinary skill in the art at the time the invention was made to modify the transcription –targeted DNA deamination method of Chaudhuri et al and include AID enzyme which modulates the alkylated DNA differentially of Bransteitter et al with a reasonable expectation of success.

An artisan would have been motivated to modify the transcription –targeted DNA deamination method Chaudhuri et al and include AID enzyme which modulates the alkylated DNA differentially of Bransteitter et al with the expected benefit of using AID enzyme, which differentially modifies single stranded DNA containing 5-methyl cytosine than cytosine and target the single stranded DNA region at the transcription site to initiate somatic hypermutation as taught by Bransteitter et al (Fig. 4b, pg. 4106, column 1, paragraph 4, pg. 4106, column 2, paragraph 3), thus enhancing the utilities of the transcription –targeted DNA deamination method of Chaudhuri et al for better understanding of somatic hypermutation in antibody diversification.

Conclusion

23. No claims are allowed

24. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Narayan K. Bhat whose telephone number is (571)-272-5540. The examiner can normally be reached on 8.30 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram R. Shukla can be reached on (571)-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For

more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Narayan K. Bhat/

Examiner, Art Unit 1634

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